IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Ketelslegers, et al.
App. No	:	10/593,372
Filed	:	January 12, 2007
For	:	PROGNOSTIC METHODS FOR CONGESTIVE HEART FAILURE
Examiner	:	James Grun
Art Unit	:	1641
Conf No.	:	8858

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Dr. Gerhard Hawa , declare as follows

1. I have expertise in interpretation of diagnostic data using ET, BigET and NT-proANP in cardiovascular diseases (see publication list, appendix). I am familiar with the Office Action of July 9, 2010 and the references cited therein.

2. Selvais et al. (2000) show survival of patients with congestive heart failure (CHF) in relation to the combination of ET-1 and N-ANP (1-25). Our key findings are (1) that "ET-1" and "Big-ET-1" are not interchangeable, and (2) the unexpected prognostic power of the association of Big-ET-1 with other markers, which was unknown in the prior art.

3. Applied to long term survival prognosis, which is a clinically relevant parameter, ET-1 and Big-ET-1 are not equivalent. When the observation is extended over a long period of time in patients with severe congestive heart failure (NYHA III – IV), measuring only ET-1, does ***NOT***

significantly segregate patients with good and poor survival. Using the Big-ET-1 (1-38) assay applied on the same population allowed to define subpopulations with poorer and better long term survival, with a high degree of significance (P for log-rank χ^2= 0.0064; P for Wilcoxon χ^2: 0.0127). This is shown in Table 1 and Figs 1 and 2 (Appendix). The difference between both assays becomes marked after 60 months of observation, a period of time never explored in previous studies. See Figs 1 and 2.

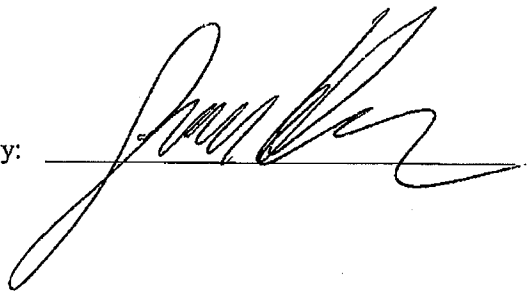
4. Differences between the outcomes of measuring BigET-1 and ET-1 are especially pronounced when applied to patients with mild CHF (NYHA I-II): Tables 2 and 3; Figs 3 and 4. To my knowledge, such robust comparison of ET-1 and Big-ET-1 applied to severe CHF or mild CHF for a prolonged follow-up is unique and necessary for the design of valid algorithms as claimed in the patent application. The Big-ET-1 immunoassay used here does not measure ET-1, because one of the antibodies used in the ELISA binds an epitope not present in ET-1. For the complete 196 months follow-up, the parameter ET offers no significant predictive value (see Table 3). That is, the analyte ET-1 is not suitable to segregate patients with mild CHF into those with good and those with poor prognosis. For a level of 3.3 pg/ml of ET, representing the upper quartile, the lack of segregation can be seen in the Kaplan-Meier survival curves of Fig 4. Similar results were obtained for the median quartile.

5. It must be emphasized that whereas the above-referenced application deals with severe **or** mild CHF, in the paper of Selvais et al a composite of mild **and** severe CHF patients is studied, which makes it impossible to distinguish the value of ET-1 and Big-ET-1. Moreover, Selvais et al used a Big-ET-1 RIA, which recognizes Big-ET (22 – 38), but has some cross-reaction with ET-1. The sandwich Elisa used for BigET-1 (1-38) is absolutely specific for Big-ET-1 (1-38) and is therefore the assay of choice. It is thus clear that only the specific assay Big-ET-1 (1- 38), is able to discriminate patients with different survivals in the class of severe CHF, *which is a unique finding that has never been described, but is embedded in the patent application.* This result is at variance with the report of **Rousseau et al** probably because of the extended observation times in the present update. Prior art does not report this finding.

6. When ET-1 was combined with the various forms of the precursor of NT-ANP, a subgroup with a low survival can be defined (~11 months), when both biomarkers are above their optimal cut-offs. However, there is almost no discrimination between the cases with both markers below or one of them below the cut-offs: Fig 5. *In contrast,* the association of Big-ET-1 (1-38) with NT-ANP (1-98), was able to provide the best discrimination between the 3 subgroups: both above, both below and one of the biomarkers below the cut-offs (Fig 6). These results are at variance with those of Selvais et al, because in their studies patients with mild and severe disease were not studied separately.

7. Also segregation of patients with mild CHF (NYHA I-II) into groups with good and poor prognoses can be improved by using certain two parameters in combination. This can be seen in Table 4, where the 50% and 75% survival estimations are presented. Although the parameters ET-1 and NT-proANP segregate patients with good and poor prognosis, the differences in 50% survival rates are not statistically significant. This is in contrast to the association of the parameters Big-ET-1 and NT-proANP. Therefore Big-ET-1 is preferred over ET-1 as parameter. The Kaplan-Meier curves presented in Figures 7 also show these findings.

8. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; an that,false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: 8th Nov., 2010 By: 

Publications

Circulating NT-proCNP predicts sepsis in multiple-traumatized patients without traumatic brain injury.
S Bahrami, L Pelinka, A Khadem, S Maitzen, G Hawa, M van Griensven, and H Redl
Crit Care Med, Jan 2010; 38(1): 161-6

Plasma N-terminal pro-B-type natriuretic peptide concentration helps to predict survival in dogs with symptomatic degenerative mitral valve disease regardless of and in combination with the initial clinical status at admission.
F Serres, JL Pouchelon, L Poujol, HP Lefebvre, C Trumel, T Daste, CC Sampedrano, V Gouni, R Tissier, G Hawa, and V Chetboul
J Vet Cardiol, Dec 2009; 11(2): 103-21.

Association of plasma N-terminal pro-B-type natriuretic peptide concentration with mitral regurgitation severity and outcome in dogs with asymptomatic degenerative mitral valve disease.
V Chetboul, F Serres, R Tissier, HP Lefebvre, CC Sampedrano, V Gouni, L Poujol, G Hawa, and JL Pouchelon
J Vet Intern Med, Sep 2009; 23(5): 984-94.

Clinical value of a competitive NT-proBNP enzyme immunoassay compared to the Roche NT-proBNP platform.
A Hammerer-Lercher, A Griesmacher, G Polzl, N Brinskelle-Schmal, J Mair, M Frick, and G Hawa
Clin Chem Lab Med, Jan 2009; 47(10): 1305-8

N-terminal pro A-type natriuretic peptide but not N-terminal pro C-type natriuretic peptide concentrations are related to cardiac diseases in infants.
A Hammerer-Lercher, B Puschendorf, R Sommer, J Mair, G Tulzer, E Lechner, G Hawa, S Maitzen, and W Woloszczuk
Clin Chim Acta, May 2008; 391(1-2): 118-9.

Natriuretic peptides correlate between newborn twins but not between twins and their mothers.
A Hammerer-Lercher, B Puschendorf, R Sommer, J Mair, G Tews, O Shebl, G Hawa, S Maitzen, and W Woloszczuk
Clin Chim Acta, Feb 2007; 377(1-2): 279-80.

Sandwich ELISA for proANP 1-98 facilitates investigation of left ventricular dysfunction.
A Missbichler, G Hawa, N Schmal, and W Woloszczuk
Eur J Med Res, Mar 2001; 6(3): 105-11.

Enzyme immunoassays for fragments (epitopes) of human proatrial natriuretic peptides.
E Hartter, S Khalafpour, A Missbichler, G Hawa, and W Woloszczuk
Clin Chem Lab Med, Jan 2000; 38(1): 27-32.

Table 1 Prognostic value for survival derived from Kaplan-Meier curves in 37 patients with severe (NYHA III-IV) CHF; classification is based on the biomarkers considered individually; cut-offs are as defined in the patent application

Biomarkers and assay		Cut-off	Classification based on cut-off L: below cut-off H: above cut-off	50 % deaths (95 % limits) MONTHS	Time to 25 % deaths (75% survival estimation) MONTHS	Number of deaths during study period	Prob.>Khi square Log-rank	Prob.>Khi square Wilcoxon
ET-1	In house RIA	8.4 pg/ml	L	31 (10-54)	10	21	0.2913 Not significant	0.0896 Not significant
			H	11 (3-18)	4.5	13		
Big-ET-1 (1-38)	2-sites Elisa	4.0 fmol/ml	L	31 (11-62)	11	20	0.0064	0.0127
			H	11 (3-16)	4	14		
Big ET-1 (22-28)	In-house RIA	13.8 pg/ml	L	31 (8-54)	8	20	0.1328 Not significant	0.2754 Not significant
			H	16 (4.5 – 22)	7	14		

Fig. 1. CHF NYHA III-IV
A: ET-1 < 8.4 pg/ml; B: ET-1 > 8.4 pg/ml

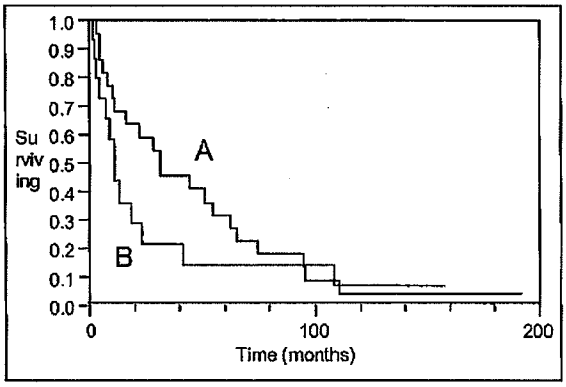


Fig. 2. CHF NYHA III-IV
A: Big-ET-1 (1-38) < 4.0 fmol/ml; B: Big-ET-1 (1-38) >4.0 fmol/ml

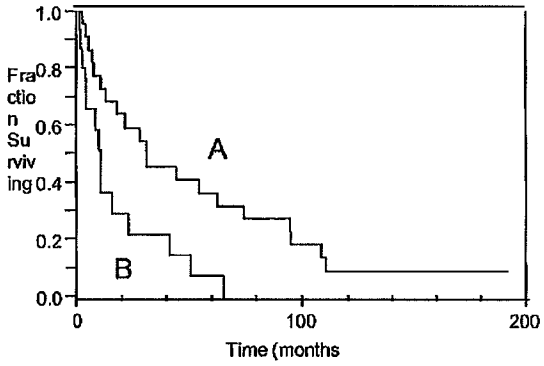


Table 2 Prognostic value for survival derived from Kaplan-Meier curves in 36 patients with mild (NYHA I-II) CHF; classification is based on the biomarkers considered individually; cut-offs are as defined in the patent application

Parameter	Method	Values segregating NYHA I and II patients with good and poor survival prognosis		
		(A) Cut-Off level	(B) Fold change of the cut-off value relative to level of the analyte in healthy people	(C) 95% confidence limits of (B)
Big-ET-1 (1-38)	Sandwich ELISA	1.8 fmol/ml	2.0	1.6 – 2.4
ET-1	RIA	None	None	None

Table 3: Comparison of Kaplan-Meier survival curve characteristics for the parameters BigET-1 and ET-1 in patients with mild CHF (NYHA I-II)

Analyte	*p* value for the difference between the samples above and below the cut-off value (chi square; log ranked)	Months for 50% survival of patients with analyte concentrations below the cut-off value
Big-ET-1	0.007	82
ET-1	Not significant	Does not apply

Fig 3: Segregation of patients with mild CHF based on the cut-off value of 1.8 fmol/ml of Big-ET-1 using the sandwich ELISA. The upper line "A" is the Kaplan-Meier survival curve of patients having a concentration of the analyte < 1.8 fmol/ml, the lower line "B" the curve for patients presenting with a concentration of the analyte > 1.8 fmol/ml.

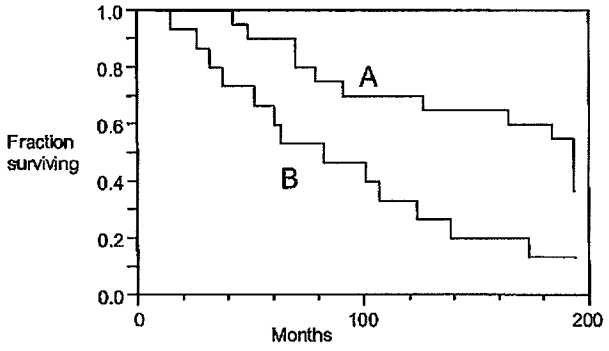


Fig 4: Kaplan-Meier survival curves of patients presenting with a plasma concentration of the analyte ET above (thin line) or below (thick line) 3.3 pg/ml.

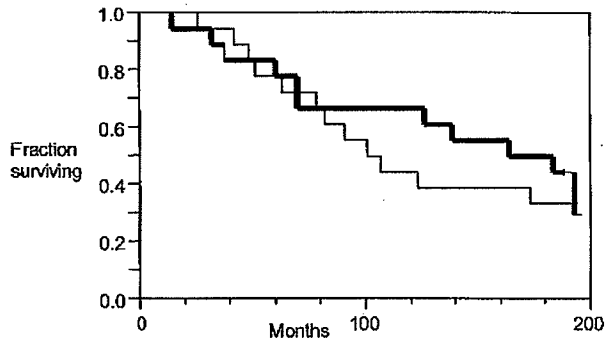


Fig.5. CHF NYHA III-IV
A: ET-1 and NT-ANP (1-98) < cutoffs
B: ET-1 OR NT-ANP (1-98) < cutoffs
C: ET-1 and NT-ANP (1-98) > cutoffs

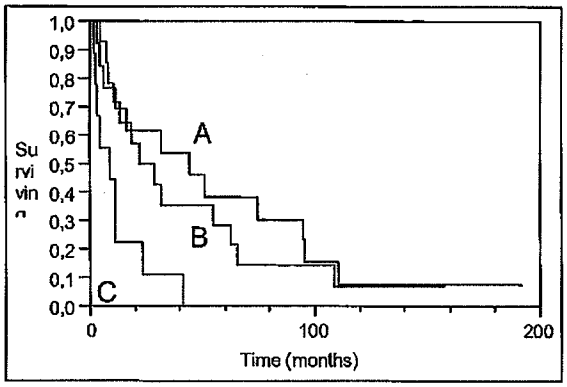
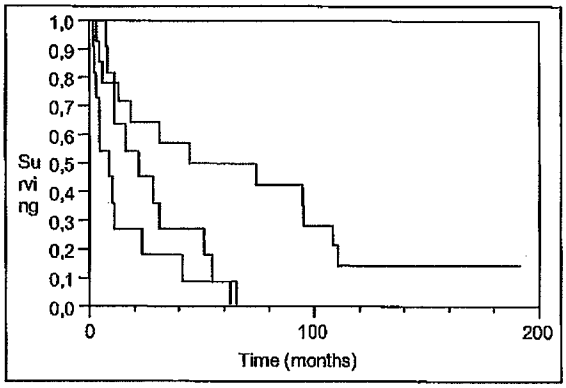


Fig.6. CHF NYHA III-IV
Upper blue line: Big-ET-1 (1-38) and NT-ANP (68-98) < cutoffs
Middle green line: Big-ET-1 (1-38) OR NT-ANP (68-98) < cutoffs
Lower red line: Big-ET-1 (1-38) and NT-ANP (68-98) > cutoffs



Analyte 1 (assay)	Analyte 2 (assay)	Analyte 1 *and* 2 below or above cut-off	Months to 50 % deaths (range based on 95 % limits)	Months to 25 % deaths	*P* value
Big-ET-1 (Sandwich ELISA)	NT-proANP (Sandwich ELISA)	Below	193 (78-194)	164	0.0162
		Above	82 (32-123)	38	
ET-1 (RIA)	NT-proANP (Sandwich ELISA)	Below	193 (60-163)	164	Not significant
		Above	91 (41-123)	51	
ET-1 (RIA)	NT-proANP (RIA for epitopes within amino acids 1-25)	Below	192 (69-193)	126	Not significant
		Above	104 (26-123)	51	
ET-1 (RIA)	NT-proANP (RIA for epitopes within amino acids 68-98)	Below	192 (70-193)	178	Not significant
		Above	100 (51-123)	63	

Table 4 Prognostic values for survival in patients with mild CHF (NYHA I-II) using markers in combination; derived from Kaplan-Meier statistics

Fig 7 Segregation of patients with mild CHF based on the cut-off value of 3293 fmol/ml for the parameter NT-proANP and the cut-off of 1.76 fmol/ml for the parameter BigET. The upper line "A" is the Kaplan-Meier survival curve of patients having a concentration of the analyte NT-proANP < 3293 fmol/ml and a concentration of the analyte BigET <1.76 fmol/ml; the lower line "B" is the curve for patients presenting with a concentration of the analytes > 3293 fmol/ml and > 1.76 fmol/ml, respectively